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Intercellular Adhesion Molecule-1, Vascular Adhesion Molecule-1, Interlukin1-β, and C - reactive protein Levels in Iraqi Patients with Type 2 Diabetes Mellitus

[1]Shatha Abdul Wadood, [2]Refif Al Shawk, [3]Hala Baher
[*1] *Department of Chemistry, College of Science, University of Baghdad, Iraq*
[2] *Specialist in immunology, National Diabetes Center for Treatment and Research, Al-Mustansiriya University, Iraq.*

Abstract : Diabetes mellitus (DM) is considered an inflammatory process with systemic involvement of the vascular tree. Asymptomic coronary artery disease is common in diabetic patients and is a strong predictor for future adverse coronary vascular events as well as early death. The objective of this study was to determine serum levels Intercellular adhesion molecule-1(ICAM-1), Vascular adhesion molecule-1(VCAM-1), interleukin 1- β (IL1- β), and C- Reactive protein (CRP) in relation to some markers indicative of type II diabeticT2DM. Methods: 50 patients in early stage of T2DM with no cardiovascular history and 30 healthy subjects were enrolled in this study. All anthropometrical indexes were measured in two groups. Laboratory investigations including: Fasting blood sugar (FBS) and lipid profile were measured by enzymatic colorimetric methods. Serum insulin, ICAM-1, VCAM-1, IL1- β, and CRP were measured by enzyme linked immunosorbent assay ELISA. Results: The levels of FBS (195.94± 12.178mg/dL), Insulin (18.627± 1.224µIU/ml). VCAM-1 (2017.3± 108.908 ng/dl), IL1-β (29.559 ± 1.225 pg/ml), CRP (23.989± 2.526 mg/l), and lipid abnormality, were highest in diabetic patients with significant differences ($P<0.05$) when compared with those of control group, while ICAM show no significant difference (57.620±0.960 ng/dl). Positive significant correlation was found between VCAM and CRP($r=0.415$, $P= 0.01$). Atherogenic Index of Plasma (AIP) show no significant differences with ICAM,VCAM, CRP, and IL1- β. Conclusions: This study reveals that even first-time diagnosis of T_2DM, patients with higher insulin resistance and abnormal lipids, have elevated endothelial dysfunction markers and CRP, which may up-regulate cardiovascular disease progression.

Keywords: C - reactive protein, Insulin resistance, Intercellular adhesion molecule-1, Interleukin 1- β, Lipid profile, Type 2 diabetes mellitus, Vascular adhesion molecule-1.

I. Introduction

Diabetes is one of the most important health and socioeconomic problem of developed countries with a growing prevalence affecting more than 4% of population. While type 1 diabetes occurs most often in children and young people and is associated with an autoimmune process destroying pancreatic beta cells, type 2 diabetes mellitus (T2DM) seems to be closely related to obesity and endocrine activity of adipose tissue. The relationship between increased body weight and waist-hip ratio (WHR) and the incidence of impaired glucose tolerance, dyslipidemia (especially hypertriglyceridemia) and hypertension was first precisely described in detail in population-based studies in the early 1980s.

This constellation of characteristic symptoms was defined as the metabolic syndrome (MetS) and was soon recognized as the main cause of global epidemic and cause of death due to diabetes and cardiovascular disease. Nevertheless, these reports did not fully explain the effect of adipose tissue on glucose metabolism [1].
Obesity is associated with endothelial dysfunction which develops before the onset of diabetes. In type 2 diabetes the development of vascular complications may be closely related to endothelial dysfunction. It seems that endothelial dysfunction in obesity may be related to insulin resistance [2].

The molecular mechanisms of insulin activity may be impaired also by the induction of chronic low-grade inflammation in adipose tissue. Adipocytes synthesize substances with chemotactic and adhesive properties, e.g., monocyte chemotactic protein-1 (MCP-1), vascular and intercellular adhesion molecules (VCAM, ICAM), which enhance the influx of lymphocytes and monocytes. Activated macrophages and adipocytes produce large amounts of proinflammatory factors, especially TNF- α, IL-1 β and IL-6[3].

Cell Adhesion Molecules (CAMs) are proteins, with a molecular weight of 95–110 kDa, located on the cell surface involved in binding with other cells or with the extracellular matrix (ECM) in the process called cell adhesion. In essence, cell adhesion molecules help cells stick to each other and to their surroundings [3].
These proteins are typically transmembrane receptors and composed of three domains: an intracellular domain that interacts with the cytoskeleton, a transmembrane domain, and an extracellular domain that interacts either

with other CAMs of the same kind (hemophilic binding) or with other CAMs of the extracellular matrix (hetrophilic binding) [4].

ICAM-1 also known as CD54 (Cluster of Differentiation 54) is a protein that in humans is encoded by the ICAM1 gene [5, 6], which is a type of intercellular adhesion molecule continuously present in low concentrations in the membranes of leukocytes and endothelial cells. Upon cytokine stimulation, the concentrations greatly increase. ICAM-1 can be induced by interleukin-1 (IL-1) and tumor necrosis factor (TNF) and is expressed by the vascular endothelium, macrophages, and lymphocytes. ICAM-1 is a ligand for Lymphocyte Function-Associated Antigene-1 LFA-1 (integrin), a receptor found on leukocytes [7]. When activated, leukocytes bind to endothelial cells via ICAM-1/LFA-1 and then transmigrate into tissues [8].

Vascular cell adhesion protein 1 also known as vascular cell adhesion molecule 1 (VCAM-1) or cluster of differentiation 106 (CD106) is a protein that in humans is encoded by the VCAM1 gene which contains six or seven immunoglobulin domains, and is expressed on both large and small blood vessels only after the endothelial cells are stimulated by cytokines [9].

The VCAM-1 protein mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium. It also functions in leukocyte-endothelial cell signal transduction, and it may play a role in the development of atherosclerosis [10], and rheumatoid arthritis [11]. Certain melanoma cells can use VCAM-1 to adhere to the endothelium, and VCAM-1 may participate in monocyte recruitment to atherosclerotic sites. As a result, VCAM-1 is a potential drug target [12].

CRP is synthesized by the liver [13] in response to factors released by macrophages and fat cells (adipocytes)[14] so CRP is used mainly as a marker of inflammation [13].

Interleukin 1-beta (IL-1βb) is a proinflammatory cytokine that plays important roles in inflammation. However, the roles of this cytokine under physiological conditions remain to be clearly delineated. A recent study showed that IL-1β plays an important role in lipid metabolism by regulating insulin levels and lipase activity under physiological conditions [15]. A number of studies have described a positive association between IL-1β gene polymorphism and obesity, suggesting functional effects on fat mass, fat metabolism and body mass [16]. In the past decades, it had been well established that inflammatory cytokines including IL-1β play a critical role in the pathogenesis of type 1 diabetes [17], although its role in type 2 diabetic are still not completely elucidated[18]. It is necessary to determine the role of Il-1b in insulin action and its secretion of pancreatic beta cells.

The aim of this study is to estimate the levels of some adhesion molecules, IL1-β and CRP in sera of newly diagnosed patients with T2DM and find their correlation with insulin resistance.

II. Methods

In the present study, 50 patients (28 males and 22 females) in early stage of T2DM (1 month to 36 months) were selected from National Diabetes Center for Treatment and Research, Al-Mustansiriya University, based on their medical history according to American Diabetes Association ADA [16] criteria. All T2DM patients were diagnosed from October 2012 to March 2013 with the ages ranging from 17 to 65 years. Thirty healthy volunteers (11 males and 19 females) with the same age range were chosen as control group. All participants gave written informed consent and all subjects were informed about the study and signed informed this forms. T2DM patients and volunteers were excluded if they had history or even manifestation of cardiovascular disease, peripheral vascular disease, coagulation disorders, neuropathy, nephropathy, insulin therapy, psychiatric illness, smoking and any acute or chronic disease.

2.1 Anthropometrical indexes

Weight and height of participants were determined in light clothing and without shoes. Portable calibrated electronic weighing scale and portable measuring inflexible bars were used. Body Mass Index (BMI) as weight (in kilograms) divided by height square (in meters), waist to hip ratio (WHR), and waist to height ratio (WHtR) were calculated.

All of the blood samples were drawn after overnight fasting and samples were kept at −80° C for subsequent assay.

2.2 Analytical methods

Lipid profile (total cholesterol Cho, very low density lipoprotein VLDL-C, triglyceride TG, low density lipoprotein LDL-C, high density lipoprotein HDL-C), and fasting blood sugar were measured by enzymatic colorimetric methods with commercially available kits (SPINREACT Company, USA, Randox Company, U.K.). Insulin was measured by enzyme linked immunosorbent assay (ELISA) (DRG Company, Germany). HOMA insulin resistance (HOMA-IR) was calculated using HOMA2 calculator. VCAM-1, ICAM-1, IL1-β and CRP were measured by enzyme linked immunosorbent assay (ELISA) kits (RayBiotech. Company, USA). All experimental procedures involving human participants were conducted with due attention to the

guidelines approved by the research ethical committee at National Diabetes Center for Treatment and Research at Al-Mustansiriya University.

2.3 Statistical analysis

All statistical analyses in this study were performed using IBM SPSS version 21.0 for windows (statistical package for social science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard error of variables. The significance of difference between mean values was estimated by two tailed student T-Test. Significance was assumed for P values less than 0.05. Pearson Correlation analysis was used to test the liner relationship between parameters.

III. Results

Table 1 show the demographic and biochemical parameters of T2DM patients and normal controls. Data were expressed as mean ± SE. Baseline characteristics such as age, body weight, BMI, WHR, and WHtR did not differ between two groups ($P \geq 0.05$).

FBS, insulin, β cell%, S%, TG, and VLDL in T2DM patients group were statistically significant than the group of healthy subjects group. As it evident there were no significant difference in HOMA2-IR, LDL, total cholesterol, and HDL between patients and control groups.

Table 2 show that serum VCAM-1, IL1- β, and CRP levels were found to be elevated significantly in patients with T2DM when compared with that of control (VCAM 2017.3± 108.908 vs. 1636.033± 72.345 ng/dl, P =0.0046), (IL1- β 29.559 ± 1.225 vs. 19.872± 1.882 pg/ml, P < 0.0001), and (CRP 23.989 ± 2.526 vs. 7.057 ± 0.910 mg/l, P < 0.0001), while ICAM show no significant difference between patients and control groups (57.620±0.960 vs. 60.055± 1.085, P = 0.091).

Pearson correlation, as shown in Table 3, was made for 38 patients with IR more than 2. CRP show a significant positive correlation with VCAM (r= 0.415 P = 0.01), FBS (r= 0.359 P = 0.027), and LDL (r= 0.355 P = 0.029), while a negative correlation with β cell% (r= -0.331 P = 0.043) and VLDL(r= - 0.352, P = 0.03) were found. Serum IL-1b was found to be positively associated with triglyceride (r= 0.338, P= 0.038). No correlation with insulin resistance was found in diabetic patients.

No significant difference was found between atherogenic index (AIP) [Log (Triglycerides/HDL-Cholesterol)] and VCAM, ICAM, and CRP as shown in Table 4.

IV. Discussion

Our study findings showed higher fasting glucose and insulin resistance in diabetic patients compared to non-diabetic subjects, while serum sensitivity and beta cell function in these patients were significantly lower than those without diabetes. These finding have been reported repeatedly by previous study [19].

Diabetes, characterized by chronic hyperglycemia, is associated with significant morbidity due to long-term complications, such as diabetic nephropathy, atherosclerosis, and hypertension. Endothelial dysfunction, by accelerating glycosylation or sorbitol pathways, is regarded as a key event in the development and progression of atherosclerosis and thought to be the major cause of vascular disease due to hyperglycemia [20].

Endothelial dysfunction is a key, early, and potentially reversible event in atherogenesis that is commonly present in human diabetes [21-23] and plays a key role in the pathogenesis of diabetic vasculopathies [24]. Several mechanisms may cause or contribute to endothelial dysfunction in diabetes. These include hyperlipidemia, oxidative stress, oxidized LDL (oxLDL), insulin resistance, formation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), and hyperglycemia [25 –27].

Diabetes is known to have higher serum insulin concentration and is a metabolic disease that occurs when pancreatic islets fail to produce sufficient insulin and/or the sensitivity of glucose-metabolizing tissues to insulin decreases [28]. In other words, the pathophysiological hallmarks of T2DM are insulin resistance and beta cell dysfunction [29]. In the present study a highly significant difference in insulin sensitivity was observed in T2DM as compared to controls. It is reported that mechanisms of islet β cell failure are different in the progression of T1DM and T2DM [28]. In individuals without diabetic, insulin secretion of beta cells is linked to peripheral insulin sensitivity through a postulated negative feedback loop that allows the beta cells to compensate for any change in whole body insulin sensitivity by a proportionate and reciprocal change in insulin secretion [30]. It is generally accepted that insulin resistance is the primary defect and that pancreatic beta cell dysfunction occurs later that contributes to the progression of diabetes. However, a number of studies conducted in Asian populations have demonstrated the dominant role of beta cell dysfunction in the pathogenesis of T2DM [31, 32]. Beta cells dysfunction is mainly destructed by autoimmune-mediated apoptosis, leading to the loss of insulin production. Inflammatory cytokines play crucial roles in this process [33].

Increased levels of CRP have been significantly associated with unfavorable outcomes [34, 35]. Decreased insulin sensitivity may lead to enhanced CRP expression by counteracting the physiological effects of insulin on hepatic acute-phase protein synthesis [36, 37].

We observed significant increase in CRP levels of T2DM patients group when compared with that of control group. In addition to being a marker of disease presence, CRP has been found to bind to endothelial cell receptors promoting apoptosis, and it has been shown to colocalize with oxidized LDL in atherosclerotic plaques. CRP also stimulates endothelial production of pro-coagulant tissue factor, leukocyte adhesion molecules, and chemotactic substances and inhibits endothelial cell nitric oxide (NO) synthase (eNOS), resulting in abnormalities in the regulation of vascular tone [38].

CRP has been demonstrated to increase the expression of ICAM-1, VCAM-1, and MCP-1 in a concentration-dependent fashion [39, 40]. Likewise, CRP has been demonstrated to facilitate native LDL uptake into macrophages, an important step in foam-cell formation [41].

Hyperglycemia acutely increases circulating cytokine concentrations [42]. HDL-cholesterol down regulates expression of adhesive molecules on the surface of vascular endothelium [43] and inhibits platelet aggregation and thus has anti-inflammatory and antithrombotic properties [44].

Our result show that the endothelial dysfunction markers VCAM-1, ICAM-1 levels were higher in diabetic patients than healthy group, which are quite similar to other studies in diabetes [45,46] and cardiovascular disease [47, 48]. The adhesion molecule VCAM-1, ICAM-1 are established markers for endothelial dysfunction and they represent major receptors controlling the influx of monocytes and other inflammatory cells into the arterial wall, their expression is considered as a hallmark in the etiology of atherosclerosis [49, 50].

In present study, we also observed that serum IL-1β concentrations were significantly higher in patients group than in control group. Proinflammatory cytokines secreted by adipose tissue and the other tissues can cause insulin dysfunction in adipose tissue, skeletal muscle and liver by inhibiting insulin signal transduction. Accumulating evidence indicates that diseases related to metabolic syndrome are characterized by abnormal cytokine production, including elevated circulating IL-1β, increased acute-phase proteins, e.g., CRP [51] and activation of inflammatory signaling pathways [52]. IL-1β plays an important role in lipid metabolism by regulating insulin levels and lipase activity under physiological conditions. A number of studies have described a positive association between IL-1β gene polymorphism and obesity, suggesting functional effects on fat mass, fat metabolism and body mass [16,17]. Recent evidence has shown that IL-1β plays a role in various diseases, including autoimmune diseases such as inflammatory bowel diseases and type 1diabetes, rheumatoid arthritis, as well as in diseases associated with metabolic syndrome such as atherosclerosis, chronic heart failure and type 2diabetes [18]. IL-1β production and secretion from pancreatic islets have also been reported [19].

Insulin has been shown to suppress NF-B binding activity, reactive oxygen species (ROS) generation, and p47phox expression and to increase IB expression in mononuclear cells (MNCs) as well as to suppress plasma concentrations of intercellular adhesion molecule-1 and monocyte chemotactic protein-1 [53, 54].
In addition, insulin suppresses AP-1 and Egr-1, 2 proinflammatory transcription factors and their respective genes, matrix metalloproteinase-9, tissue factor (TF), and PAI-1 [55, 56, 57]. Thus, insulin has a comprehensive anti-inflammatory effect and in addition has an antioxidant effect, as reflected in the suppression of ROS generation and p47phox expression [58, 59]. Two further pieces of evidence demonstrating the anti-inflammatory action of insulin have emerged recently. First, the treatment of type 2 diabetes with insulin for 2 weeks caused a reduction in CRP and monocyte chemotactic protein-1[60]. Second, the treatment of severe hyperglycemia associated with marked increases in inflammatory mediators with insulin resulted in a rapid marked decrease in the concentration of inflammatory mediators [61].

In a rat model in which inflammation was induced with endotoxin, insulin suppressed the concentration of these inflammatory mediators, including interleukin (IL)-1, IL-6, macrophage migration inhibition factor (MIF), and tumor necrosis factor (TNF) [62].

Insulin also suppressed the expression of the proinflammatory transcription factor CEBP and cytokines in the liver of these animals. Similar reductions in inflammatory mediators were observed in rats with thermal injury treated with insulin [63].

Finally, insulin has been shown to suppress the increase in cytokine concentration in pigs challenged with endotoxin [64]. Another insulin action that regulates vascular function is stimulation of the expression of vascular cell adhesion molecule (VCAM)-1 and E-selectin on endothelium. MAP-kinase–dependent signaling pathways (but not PI 3-kinase pathways) regulate these functions of insulin [65]. This may explain the lack of association between ICAM and VCAM, and between ICAM and CRP in this study since the patients were newly diagnosed and insulin may act as anti-inflammatory agent caused by hyperglycemia.

The molecular mechanisms of insulin activity may be impaired also by the induction of chronic low-grade inflammation in adipose tissue. Adipocytes synthesize substances with chemotactic and adhesive properties, e.g., monocyte chemotactic protein-1 (MCP-1), and vascular and intercellular adhesion molecules (VCAM, ICAM), which enhance the influx of lymphocytes and monocytes. Activated macrophages and adipocytes produce large amounts of proinflammatory factors, especially TNF- α, IL-1 β and IL-6 [2].In this study we found no correlation between obesity and intercellular adhesion molecules levels leading us to

conclude that obesity has no role in the inflammation state (high CRP levels) observed in the cases under study which may be due to hyperglycemia.

Diabetes increases the risk for atherogenesis via deleterious effects on the vessel wall. The vascular abnormalities leading to atherosclerosis in patients with diabetes may be evident before the diagnosis of diabetes, and they increase with duration of diabetes and worsening blood glucose control.

V. Conclusion

The preclinical phase of this disease lasts many decades, and this provides an opportunity for the pre-symptomatic detection of high-risk subjects. In diabetes type 2 we found elevated levels of ICAM-1, VCAM, IL-β and CRP, which can confirm an endothelial dysfunction in patients group.

We believe that evaluation of ICAM-1, VCAM-1, and CRP together can help early and better to identify an increased likelihood of cardiovascular disease in the diabetic population. These biomarkers might represent a useful and cost-effective means of identifying the subset of diabetic patients that require stress testing in order to be diagnosed and treated best. Therapeutic strategies focused on decreasing serum levels of these biomarkers may be useful to improve vascular function in DM and in other vascular pathologies characterized by a chronic inflammatory state.

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Table (1): Demographic and biochemical parameters of T2DM patients (n=50) and control group (n=30).

Parameters	Control (30) Mean (±SE)	Patients(50) Mean (±SE)	value P
Age	39.566(1.625)	42.714(1.381)	0.148
BMI	27.856(0.758)	29.218(0.720)	0.197
WHR	0.916(0.0140)	1.531(0.560)	0.277
WHtR	0.580(0.015)	0.606(0.013)	0.205
VLDL (mg/dl)	22.6(0.979)	36.26(3.309)	0.0002
LDL(mg/dl)	91.133(4.930)	94.26(5.156)	0.662
TG(mg/dl)	112.566(4.891)	166.68(14.687)	0.0009
Total cholesterol TC (mg/dl)	159.666(4.797)	172.08(5.245)	0.084
HDL –C(mg/dl)	46.8(0.980)	45.08(0.788)	0.176
FBS (mg/dl)	91.633(2.139)	195.94(12.178)	p< 0.0001
Insulin (µIU/ml)	9.861(0.452)	18.627(1.224)	p< 0.0001
HOMA2-IR	1.273(0.056)	5.752(2.468)	0.075
B Cell%	114.223(6.995)	59.642(6.187)	p< 0.0001
S %	83.49(4.123)	40.508(3.124)	p< 0.0001

Table (2): Biomarkers levels T2DM patients (n=50) and control group (n=30).

Parameters	Control(n=30) Mean(±SE)	Patients(n=50) Mean(±SE)	P value
ICAM-1(ng/dl)	60.055(1.085)	57.620(0.962)	0.091
VCAM-1(ng/dl)	1636.033(72.345)	2017.3(108.908)	0.0046
CRP (mg/l)	7.057(0.910)	23.989(2.526)	p< 0.0001
IL-1B (pg/ml)	19.872(1.882)	29.559(1.225)	p< 0.0001

Table 3: Pearson correlation analysis of (38) patients with IR > 2.

Parameters	CRP		IL1- β		VCAM		ICAM	
	r	p	r	p	r	P	r	p
BMI	0.166	0.319	0.114	0.497	0.139	0.404	0.052	0.757
WHR	-0.057	0.735	-0.111	0.507	0.160	0.338	-0.061	0.715
WHtR	0.193	0.247	0.019	0.912	0.140	0.400	0.053	0.754
VLDL-C	-0.352*	0.030	0.115	0.491	-0.178	0.285	0.162	0.332
LDL-C	0.355*	0.029	-0.249	0.131	0.290	0.077	-0.230	0.164
TG	-0.288	0.079	0.338*	0.038	-0.115	0.492	0.019	0.908
TC	0.121	0.468	-0.129	0.442	0.204	0.220	-0.175	0.294
HDL-C	0.011	0.950	-0.187	0.262	-0.184	0.270	0.115	0.492
insulin(µU/l)	-0.153	0.358	-0.122	0.465	0.023	0.889	0.117	0.483
FBS(mg/dl)	0.359*	0.027	-0.107	0.523	0.005	0.976	0.045	0.787
B cell%	-0.331*	0.043	0.033	0.843	-0.040	0.809	0.079	0.639
%S	-0.235	0.156	0.032	0.850	-0.032	0.849	-0.231	0.164
HOMA2 - IR	-0.011	0.948	-0.077	0.646	-0.102	0.543	0.039	0.817
CRP (mg/l)	1	-	-0.032	0.850	0.415**	0.010	0.058	0.732
IL-1B (pg/ml)	-0.032	0.850	1	-	-0.079	0.639	0.061	0.714
VCAM-1(ng/dl)	0.415*	0.010	-0.079	0.639	1	-	-0.047	0.779
ICAM-1(ng/dl)	0.058	0.732	0.061	0.714	-0.047	0.779	1	-

Table (4): Attherogenic index [Log (Triglycerides/HDL-Cholesterol)] and VCAM-1, ICAM-1, CRP levels of T_2DM patients (n=50).

	Atherogenic Index of categories			
	Low risk (<0.11)	Intermediate risk (0.11-0.2)	High risk (0.21+)	P
CRP(mg/L)				0.46[NS]
Range	(0.78 to33.51)	(1.91 to 29.84)	(2.29 to 61.75)	
Median	12.23	12.02	8.89	
Inter-quartile range	(5.65to20.48)	(3.94 to 20.94)	(6.16 to 10.68)	
N	23	8	18	
Mean rank	27.26	25.88	21.72	
VCAM1(ng/ml)				0.35[NS]
Range	(965 to 3839)	(1366 to 3656)	(1078 to 4222)	
Mean	1862.7	2156	2100.8	
SD	631.8	789.5	975.8	
SE	131.75	279.12	229.99	
N	23	8	18	
ICAM-1(ng/ml)				0.75[NS]
Range	(44.7 to 67.4)	(44.3 to 68.9)	(43.4 to 67.6)	
Mean	57.2	60.3	56.5	
SD	6.5	8.1	6.6	
SE	1.36	2.87	1.56	
N	23	8	18	